ABSTRACT

DISSERTATION: A Novel Method of Assessing Human Skeletal Muscle Fiber Type Specific Protein Content

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Little is known about protein profiles in slow-twitch (MHC I) and fast-twitch (MHC IIa and MHC IIx) human skeletal muscle fibers. Therefore we developed a method of assessing fiber type specific protein content across the continuum of human skeletal muscle fiber types. The method presented here combines the advantages of SDS-PAGE for fiber typing with the common Western Blot (WB) technique. Individual vastus lateralis muscle fibers (n = 264) were isolated and clipped into two portions, one for fiber-typing and one for protein identification. Following fiber type determination, WB destined fiber segments were combined into fiber type specific pools (20 fibers/pool) and assessed for GAPDH, actin, Citrate Synthase, and total p38 content. GAPDH expression was 69%, 92%, 159%, and 200% more abundant in MHC I/IIa, MHC IIa, MHC IIa/IIx, and MHC IIx pools when compared to MHC I, respectively. Inversely, Citrate synthase content was 526%, 497%, 316%, and 47% more abundant in MHC I, MHC I/IIa, MHC IIa, and MHC IIa/IIx when compared to MHC IIx, respectively. Similar to GAPDH, total p38 expression was 67% greater in MHC IIa versus MHC I fibers. These data establish a novel application of WB combined with SDS-PAGE for fiber type

specific protein analysis in human skeletal muscle. These initial results show content of particular proteins exist in a hierarchal fashion throughout the continuum of human skeletal muscle fiber types. Application of these methods will enhance our understanding of skeletal muscle health profiles among physically active and clinically based populations.